Genome-wide association and interaction studies of CSF T-tau/Aβ_{42} ratio in ADNI cohort

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Abstract—The pathogenic relevance in Alzheimer's disease (AD) presents a decrease of cerebrospinal fluid (CSF) amyloid-ß$_{42}$ (Aß$_{42}$) burden and an increase in CSF total-tau (T-tau) levels. In this work, we performed genome-wide association study (GWAS) and genome-wide interaction study (GWIS) of T-tau/Aß$_{42}$ ratio as an AD imaging quantitative trait (QT) on 843 subjects and 563,980 single nucleotide polymorphisms (SNPs) in ADNI cohort. We aim to identify not only SNPs with significant main effects but also SNPs with interaction effects to help explain "missing heritability". Linear regression method was used to detect SNP-SNP interactions among SNPs with uncorrected p-value≤0.01 from the GWAS. Age, gender and diagnosis were considered as covariates in both studies. The GWAS results replicated the previously reported AD-related genes *APOE*, *APOC1* and *TOMM40*, as well as identified 14 novel genes, which showed genome-wide statistical significance. GWIS revealed 7 pairs of SNPs meeting the cell-size criteria and with bonferroni-corrected p-value≤0.05. As we expect, these interaction pairs all had marginal main effects but explained a relatively high-level variance of T-tau/Aß$_{42}$, demonstrating their potential association with AD pathology.

Keywords: Cerebrospinal fluid (CSF), Amyloid-ß$_{42}$ (Aß$_{42}$), Total tau (T-tau), T-tau/Aß$_{42}$ ratio, GWIS, ADNI.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia and characterized by pathological results at autopsy of the accumulation of amyloid-ß (Aß) protein in senile plaques and hyper-phosphorylated tau in neurofibrillary tangles in brain (Mukaetova-Ladinska et al., 2015). The levels of two measures, cerebrospinal fluid (CSF) amyloid-ß$_{42}$ (Aß$_{42}$) and total tau (T-tau), have been shown strong promise as predictive biomarkers of the progression from mild cognitive impairment (MCI, a prodromal stage of AD) to AD (Blennow and Hampel, 2003; Pan et al., 2015). Typically, the pathogenic relevance in AD presents a decrease of CSF Aß$_{42}$ burden and an increase in CSF T-tau levels simultaneously (Li et al., 2015).

However, the emerging literatures reported that a group of individuals have never shown clinical symptoms of AD in their lifetime but detected out tauopathies and amyloid plaques at autopsy (Hohman et al., 2014). In addition, some normal cognitive individuals presented low CSF Aß$_{42}$ burden and some individuals with definitive diagnosis of AD showed high levels of CSF Aß$_{42}$ due to their lack of amyloid deposition (Fagan et al., 2006). The emergence of this situation posed challenges on discriminating individuals with AD from normal cognitive, and affected the diagnostic potential of these markers. To address this issue, a potential biomarker CSF T-tau/Aß$_{42}$ ratio demonstrated its predictive ability. It can be used to detect and measure the AD risk with cognitive decline in non-demented older adults, and individuals with higher ratio tend to have higher risk to develop AD (Fagan et al., 2007). Moreover, prior studies also showed that individuals with family history of AD had higher risk for AD than those without a family history. This indicates that the underlying genetic factors may play an important role in AD (Hohman et al., 2014).

The existing genome-wide association studies (GWAS) have analyzed Single Nucleotide Polymorphism (SNP) data and discovered a wide array of underlying genetic causes of AD and genetic associations with AD biomarkers as intermediate quantitative traits (QTs). For many conditions of complex diseases and traits, commonly used single marker analysis can identify a number of risk genetic loci, but these identified variants typically appear to explain only a modest portion of the theoretical estimates of genetic heritability (Goudey et al., 2013). One possible reason is that the univariate methods used in GWAS typically ignore the factor of underlying genetic interaction, which may contribute to the development of disease and trait variance.

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** Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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Thus, one of several putative explanations for the "missing heritability" is that the trait variance can partially be explained by the SNP-SNP interaction effects in addition to their main effects (Becker et al., 2011). Therefore, genome-wide interaction studies (GWIS) have recently gained substantial attention (J. Li et al., 2015).

In this study, we performed both GWAS and GWIS in the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort. We used the AD-associated CSF T-tau/Aß$_{42}$ ratio as QT, and tested single-marker main effects and two-marker interactions at the genome-wide level.

2. Materials and Methods

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD).

We applied for and were granted permission to use data from the ADNI cohort (http://www.adni-info.org/) to conduct genetic association and interaction analyses.

2.1. Subjects

Participants are ADNI subjects (N=843) with CSF T-tau and Aβ$_{42}$ measures and quality controlled genotyping data available at baseline. The sample included 199 cognitively normal (CN), 85 significant memory concern (SMC), 239 early mild cognitive impairment (EMCI), 207 late mild cognitive impairment (LMCI), and 113 AD participants. Table 1 shows selected participant characteristics at the baseline, which is the time point studied in this work.

2.2. Quality control of genotyping data

The genotyping data of the ADNI-1, ADNI-GO and ADNI-2 cohorts were collected using either the Illumina 2.5M array (a byproduct of the ADNI whole genome sequencing sample) or the Illumina OmniQuad array (Saykin et al., 2010; Shen et al., 2010; Shen et al., 2014), and were downloaded from the LONI website (http://adni.loni.usc.edu). For the present analyses, we included SNPs that were present on both arrays.

Quality control (QC) was performed using the PLINK software (version 1.90) (Purcell et al., 2007). SNPs were removed from the analysis if any of the following criteria were not satisfied: (1) SNPs on chromosome 1-22; (2) call rate per SNP $\geq$ 95%; (3) minor allele frequency $\geq$ 5% (1,845,510 SNPs were removed based on Criteria 1, 2 and 3); and (4) Hardy-Weinberg equilibrium (HWE) test of $p \geq 10^{-6}$ using CN subjects only (198 SNPs were removed). Participants not meeting any of the following criteria were removed from further analyses: (1) call rate per participant $\geq$ 90% (none); (2) sex check (1 participant was removed); and (3) identity check for related pairs (8 sibling pairs and one triplet were identified with PI_HAT $\geq$ 0.25, 9 participants (one from each pair or triplet) were randomly selected and included in this study).

Population stratification analysis was performed using EIGENSTRAT (Price et al., 2006), and confirmed using STRUCTURE (Pritchard et al., 2000). It yielded 89 participants who did not cluster with the remaining subjects and with the CEU HapMap samples who are primarily of European ancestry (non-Hispanic Caucasians). These 89 participants were excluded from the analysis. Among the remaining 1,079 subjects, only 843 subjects have both genotyping data and phenotypes (T-tau and Aß$_{42}$) after quality control (QC), and thus the other 236 participants were excluded.

After QC, 843 subjects and 563,980 SNPs remained for the subsequent genome-wide association and interaction analyses.

2.3. CSF T-tau/Aß$_{42}$ biomarker

In our study, the CSF levels of T-tau and Aß$_{42}$ at baseline were used. The methods for CSF acquisition and biomarker measurement have been reported previously (Hampel et al., 2010; Jagust et al., 2009; Shaw et al., 2009). For this analyses, the Aß$_{42}$ and T-tau data were log-transformed to better approximate normality in distribution (Dickerson et al., 2013), and the values greater or smaller than 4 SDs (standard deviation) from the mean value of Aß$_{42}$ and T-tau were regarded as extreme outliers and excluded from the analyses.

Table 1. The demographic and clinical characteristics of 843 ADNI participants at baseline studied in this work.

		CN (N=199)	SMC (N=85)	EMCI (N=239)	LMCI (N=207)	AD (N=113)
Age (years)		74.4(7.79)	72.0(5.48)	71.4(7.30)	72.4(7.61)	75.2(8.19)
Women		96(48.2%)	50(58.8%)	102(43.6%)	83(40.1%)	45(39.8%)
Education (years)		16.21(2.82)	16.00(2.79)	16.16(2.80)	16.38(2.53)	16.40(2.56)
APOE e4 allele present		47(23.6%)	31(36.5%)	99(41.4%)	112(54.1%)	74(65.5%)
CDR–SOB		0.04(0.14)	0.08(0.18)	1.27(0.77)	1.65(0.94)	4.53(1.70)
Mini mental status examination		29.06(1.18)	29.04(1.420)	28.34(1.62)	27.54(1.75)	23.12(2.04)
Logical memory immediate recall (WMS-R)		14.42(3.00)	14.44(3.34)	11.09(2.68)	7.18(3.06)	4.15(2.70)
Logical memory delayed recall (WMS-R)		13.34(3.12)	13.29(3.31)	8.97(1.73)	3.94(2.70)	1.52(1.80)
CSF T-tau (Total tau)		69.76(31.76)	66.95(31.68)	77.66(46.97)	98.22(52.50)	126.26(54.47)
CSF Aβ$_{42}$ (amyloid-β$_{42}$)		198.09(52.87)	201.42(49.43)	184.50(51.41)	162.92(52.80)	136.90(36.25)
Quantitative Traits (**QTs**)	T-tau/Aβ$_{42}$ ratio	0.40(0.27)	0.37(0.24)	0.50(0.45)	0.70(0.47)	0.98(0.49)

Abbreviations: AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; CDR–SOB, clinical dementia rating–sum of boxes; CN, cognitive normal; SMC, significant memory concern; EMCI, early mild cognitive impairment; LMCI, late mild cognitive impairment; WMS-R, Wechsler Memory Scale-Revised. Data are number (%) or mean (s.d).

After QC, 843 valid CSF samples remained.

2.4. Method of GWAS and GWIS

GWAS was used to evaluate the SNPs main effects at the genome-wide level. Our study performed a genotypic model based GWAS using PLINK 1.90 to detect the association between SNPs and the T-tau/Aß$_{42}$ ratio with age, gender and clinical diagnosis (five values (1-5) indicating CN, SMC, EMCI, LMCI and AD respectively) as covariates. Manhattan plots and quantile-quantile (Q-Q) plots were generated using Haploview (http://www.broad.mit.edu/mpg/haploview/) and R (http://www.r-project.org) respectively.

For GWIS detecting the SNP-SNP interactions, software tool INTERSNP (Herold et al., 2009) was used for two-marker analysis. The input files of the tool were PLINK genotype files. Firstly, single-marker test was performed for GWAS as previously described. The SNPs that met the threshold (uncorrected p-value $\leq$ 0.01) were included in the subsequent interaction analysis. Linear regression model was used for an additive interaction test (full model including both additive and dominance effects plus interaction term versus reduced model that does not contain interaction terms) on all possible SNP pairs among the previous SNPs selected in first step. We detected the epistasis interactions with the T-tau/Aß$_{42}$ ratio as QT while controlling for covariates including the baseline age, gender, and clinical diagnosis. There were about 22 million unique SNP pairs to be examined, and the Bonferroni corrected p-value < 0.05 was used as the statistical significance threshold.

2.5. Post hoc analysis

We performed a hierarchical linear regression among the significant interactions, used IBM SPSS 20 to estimate the amount of variance (R^2) on the T-tau/Aß$_{42}$ level accounted for by these interaction terms. We first included the same set of covariates (age, gender, and diagnosis) in the linear model, and then included apolipoprotein *E* (*APOE*) status, the best-known AD risk gene (Akiyama et al., 1993), and two main effects of SNPs from the significant pair. Finally, we included the SNP-SNP interaction term and computed additional variance explained by interaction term. The difference in R^2 for the significant model was calculated in SPSS as $\Delta R^2 = R^2_{full}$ (full model with interaction term) – R^2_{domain} (reduced model without interaction term).

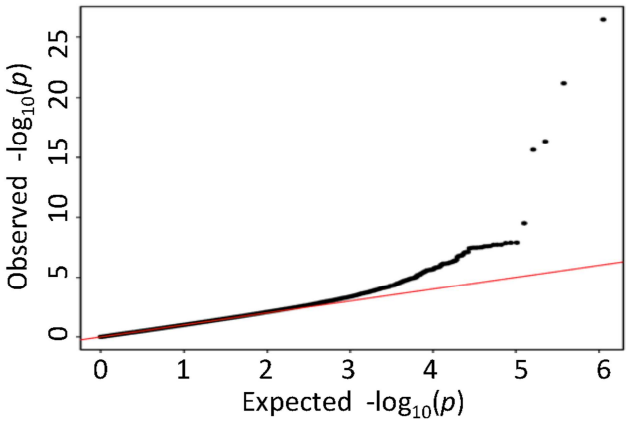

Fig. 1. Quantile–quantile (Q–Q) plot of the observed -log$_{10}$(p-values) from the GWAS of T-tau/Aß42 level versus those expected under the null hypothesis.

3. Results

3.1. Genome-wide association study results

The demographic and clinical characteristics of the 843 participants at baseline were shown in Table 1. Q-Q plot (Fig. 1) shows five evident outliers at the high end of the range, and indicates no evidence of spurious inflation. The Manhattan plot in Fig. 2 shows the same five outliers with high significance, and there are a few other significant hits shown above the red line (Bonferroni-corrected threshold p-value = 0.05).

In single-marker analyses, 24 SNPs exhibited genome-wide significance to the T-tau/Aß$_{42}$ ratio (Table 2). As expected, the most significant loci were identified on chromosome 19, including rs4420638 (p=3.50E-27) from the *APOC1* region, rs769449 (p=6.41E-22) within the *APOE* region, and rs2075650 (p=4.40E-17) and rs157582 (p=2.29E-16) within the *TOMM40* region. Other SNPs identified in this study are shown in Table 2. Table 2 also shows the variances explained by each identified SNP after controlling two sets of covariates: (1) age, gender, and diagnosis; and (2) age, gender, diagnosis,

Table 2. Identified significant genome-wide association loci with quantitative trait T-tau/Aß$_{42}$ in this study.

NO.	CHR	rs_No.	Gene	Single-marker p_value	**Corrected p-value**	R Square		No.	CHR	rs_No.	Gene	Single-Marker p_Value	**Corrected p-value**	R Square	
						Model[a]	Model[b]							Model[a]	Model[b]
1	19	rs4420638	*APOC1*	3.50E-27	**1.97E-21**	0.100	0.001	13	1	rs10127852	*LPAR3*	2.46E-08	**0.0137**	0.034	0.023
2	19	rs769449	*APOE*	6.41E-22	**3.62E-16**	0.091	0.0	14	15	rs9806191	*DAPK2*	2.56E-08	**0.0143**	0.034	0.023
3	19	rs2075650	*TOMM40*	4.40E-17	**2.48E-11**	0.071	0.0	15	11	rs7129826	*DBX1*	2.99E-08	**0.0167**	0.033	0.023
4	19	rs157582	*TOMM40*	2.29E-16	**1.25E-10**	0.068	0.001	16	21	rs11910985	*S100B*	3.17E-08	**0.0177**	0.033	0.023
5	1	rs13376197	*B3GALT2*	3.04E-10	**0.00017**	0.042	0.031	17	21	rs1981331	*S100B*	3.19E-08	**0.0178**	0.033	0.023
6	12	rs3020811	*LRP6*	1.24E-08	**0.0069**	0.030	0.026	18	3	rs9872004	*AADACL1*	3.26E-08	**0.0181**	0.033	0.023
7	9	rs2280302	*FBP1*	1.28E-08	**0.0071**	0.035	0.026	19	1	rs17027633	*ATP5F1*	3.26E-08	**0.0182**	0.033	0.023
8	9	rs2280301	*FBP1*	1.37E-08	**0.0076**	0.035	0.026	20	1	rs6662771	*SGIP1*	3.44E-08	**0.0192**	0.033	0.023
9	10	rs12265790	*ITGA8*	1.85E-08	**0.0103**	0.034	0.024	21	11	rs12797204	*DLG2*	3.71E-08	**0.0206**	0.033	0.025
10	10	rs7896076	*ITGA8*	1.87E-08	**0.0104**	0.034	0.024	22	18	rs6506440	*ARHGAP28*	7.51E-08	**0.0414**	0.032	0.026
11	10	rs11253637	*ITGA8*	1.91E-08	**0.0107**	0.034	0.024	23	2	rs6541929	*CNTNAP5*	7.64E-08	**0.0421**	0.032	0.027
12	1	rs1539581	*ATP5F1*	2.34E-08	**0.0130**	0.034	0.024	24	2	rs17267326	*CNTNAP5*	8.23E-08	**0.0453**	0.031	0.028

[a] Model: Percent of additional variance in T-tau/Aß$_{42}$ level explained by the main effect of SNPs after accounting for age, gender, and diagnosis.
[b] Model: Percent of additional variance in T-tau/Aß$_{42}$ level explained by the main effect of SNPs after accounting for age, gender, diagnosis, and the *APOE*

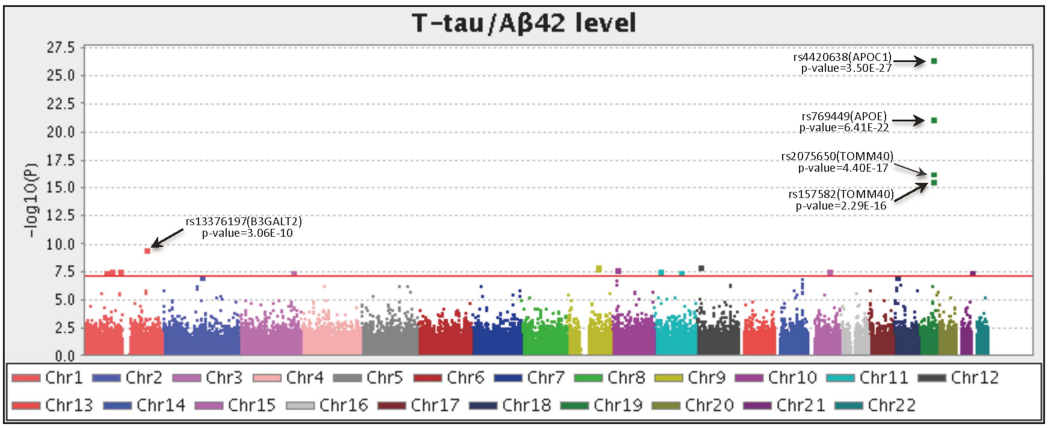

Fig. 2. Manhattan plot of the observed -$\log_{10}$(p-values) from the GWAS CSF. More than 560,000 SNPs were tested for association to T-tau/Aβ_{42} level under an genotypic model with age, gender and diagnosis as covariates. Genome-wide associations identified 22 significant SNPs (Bonferroni-corrected threshold is p-value < 0.05 and represented by the red line), and the top 4 significant SNPs were on chromosome 19 within the *APOE* and its neighboring regions.

and the *APOE e4* status. The main effects of rs4420638, rs769449, rs2075650, and rs157582 account for 10%, 9.1%, 7.1%, and 6.8% of phenotypic variance respectively while controlling for age, gender and diagnosis, but account for ≤0.1% variance after removing the *APOE e4* status. The most significant AD-risk factor *APOE e4* SNP (rs429358) accounts for 12.9% variance (Table 3). The total amount of additional variance explained by 24 identified SNPs is 11.3% after accounting for age, gender, diagnosis and *APOE* e4 status, and up to 41.6% while including all factors (age, gender, diagnosis, *APOE* and 24 SNPs).

3.2. Genome-wide interaction study results

Our two-marker interaction model considered age, gender, and clinical diagnosis as covariates. 307 pairs of SNPs showed statistically significant interaction effects on the T-tau/Aß$_{42}$ level (Bonferroni-corrected p-value<0.05). Only 7 pairs passed the cell-size criterion: all the cell sizes in 3-by-3 contingency table are required to be either more than 5 or equal to 0. The results of two-marker interaction were: rs1514061 (*PLXNA4**) - rs6467419 (*PLXNA4**), rs1514061 (*PLXNA4**) - rs4453471 (*CDH13*), rs7303599 (*ADIPOR2**) - rs7146454 (*ADSSL1**), rs7303599 (*ADIPOR2**) - rs167396 (*GSN**), rs1482548 (*INHBA**) - rs12894119 (*NIN**), rs9550406 (*MTUS2**) - rs6471951 (*RLBP1L1*), rs211953 (*CXADR*) - rs4881147 (*PITRM1**), where * indicating nearest gene proximal to the SNP. Details are available in Table 3.

3.3. Post hoc analysis

Table 3 also shows the two-marker interaction results of post hoc analysis on T-tau/Aß$_{42}$ level. Age, gender, and diagnosis were first included in the model and accounted for 17.4% of variance on the T-tau/ Aß$_{42}$ level. *APOE* status was then accounted for an additional 12.9% of variance. For each interaction, we ran a hierarchical linear regression model. We first added in the genetic main effects, and then the genetic interaction term to determine the variance associated with the interaction term alone. For rs1514061 (*PLXNA4**) - rs6467419 (*PLXNA4**), the SNPs' main effects accounted for 1.5% of variance, and the interaction term accounted for 5.1% of variance (6.6% combined). For rs1514061 (*PLXNA4**) - rs4453471 (*CDH13*), the main effects accounted for 1.9% of variance, and the interaction accounted for 4.7% of variance (6.6% combined). For rs7303599 (*ADIPOR2**) - rs7146454 (*ADSSL1**), the main effects accounted for 2.3% of variance, and the interaction term accounted for 4.2% of variance (6.5% combined). For rs7303599 (*ADIPOR2**) - rs167396 (*GSN**), the main effects accounted for 2.1% of variance, and the interaction term accounted for 4.1% of variance (6.2% combined). For rs1482548 (*INHBA**) - rs12894119 (*NIN**), the main effects accounted for 1.5% of variance, and the interaction term accounted for 3.8% of variance (5.3% combined). For rs9550406 (*MTUS2**) - rs6471951 (*RLBP1L1*), the main effects accounted for 1.0% of variance, and the interaction accounted for 3.4% of variance (4.4% combined). For rs211953 (*CXADR*) - rs4881147 (*PITRM1**), the main effects accounted for 2.4% of variance, and the interaction term accounted for 3.1% of variance (5.5% combined).

4. Discussion

In this work, we performed GWAS and GWIS of the CSF biomarker T-tau/Aß$_{42}$ ratio, using a sample of 843 subjects from the ADNI database. To our knowledge, this genome-wide study on detecting two-marker interaction is the first GWIS on the quantitative trait of the T-tau/Aß$_{42}$ level.

In single-marker analysis, we identified the SNPs in *APOE*, *APOC1* and *TOMM40* genes (Fig. 2), which showed high-level genome-wide significant associations to the T-tau/Aß$_{42}$ ratio. We also revealed 20 additional significant loci, within or proximal to *LRP6*, *S100B*, *DLG2*, *CNTNAP5*, *B3GALT2*, *FBP1*, *ITGA8*, *ATP5F1*, *LPAR3*, *DAPK2*, *DBX1*, *AADACL1*, *SGIP1*, and *ARHGAP28* genes (Table 2). The previously reported AD risk genes *APOE*, *APOC1*, and *TOMM40* were replicated in our GWAS (Supplementary Table s4). In addition, the *S100B*, *CNTNAP5*, *LRP6*, and *DLG2* genes were also reported to have pathological relevances in AD. *S100B* shows a pathological relevance for degeneration of the central nervous system in AD (Petzold et al.,2003), and overexpression of *S100B* in the neuritic plaques of AD is related to the degree of neuritic pathology in Aß plaques (Peskind et al., 2001). *CNTNAP5* encodes the protein belonging to the neurexin family functioning in the central nervous system as cell

adhesion molecules and receptors, and has been implicated as a risk factor for posterior cortical atrophy variant of AD (Schott et al., 2016). Neuronal *LRP6* mediated Wnt signaling has an impact on synaptic function and cognition, and genetic variants in the *LRP6* gene have been linked to AD risk (Liu et al., 2014). A proteomics study showed AD-dependent changes in the DLG2 level in the hippocampus, and DLG2 exhibits an early-up, late-down expression pattern during AD pathology (Hondius et al., 2016). Our exploratory GWAS nominates the others novel loci, such as *B3GALT2*, *FBP1*, *ITGA8*, *ATP5F1*, *LPAR3*, *DAPK2*, *DBX1*, *AADACL1*, *SGIP1* and *ARHGAP28*, meeting the genome-wide significance. These potential T-tau/Aß$_{42}$ related quantitative trait loci (QTLs) warrant further investigation.

SNP-SNP interaction studies may explain part of the "missing heritability". The recent studies (Shen et al., 2014) in ADNI cohort demonstrated "case-control" studies for testing epistasis interaction. In this study, we preformed two-marker interaction analyses using the T-tau/Aß$_{42}$ ratio as quantitative trait for increasing statistical power and reducing required sample sizes. Our method revealed 7 pairs of SNPs within or proximal to 11 genes meeting the criterion of the cell size either more than 5 or equal to 0 and a Bonferroni corrected threshold (corrected p-value ≤ 0.05). As we expected, the significant variants in these pairs all have marginal dominance effects, but their interactions can explain a relatively high-level variances of the T-tau/Aß$_{42}$ ratio (Table 3), and high-level AD risk. The bar charts of the QT measures across SNP-by-SNP genotype combinations are shown in Fig. 3.

In previous studies, *PLXNA4* has been reported to be associated with precise positioning of OPCs (oligodendrocyte precursor cells) in developing cerebral cortex. Then it has also suggested that *PLXNA4* does not influence APP processing or Aβ production but its isoform differentially affects tau protein phosphorylation (Jun et al., 2014) involved in AD pathogenesis, leading to neurofibrillary tangle formation and neuronal death (Wang et al., 2016). *CDH13* gene has been linked to brain function or neuropsychiatric disorders, affecting morphometry of the temporal lobes (a typical AD biomarker) (Kohannim et al., 2012). With these observations, the identified *PLXNA4-PLXNA4* and *PLXNA4-CDH13* interactions may have a potential on contributing to the tau pathway instead of Aβ.

ADIPOR2 (Adiponectin Receptor 2) is a protein coding gene, and adiponectin is the most abundant adipokine secreted from adipose tissue. Globular adiponectin has been reported to induce a pro-inflammatory response in human astrocytic cells (Chan et al., 2012; Wan et al., 2014). Aβ caused neuroinflammation plays a critical role in the development of neurodegenerative disorder in AD pathogenesis. *ADSSL1* is an Aβ toxicity modifier gene, and also an intracellular protein responsible for catalyzing the first step of de novo biosynthesis of AMP. Its genetic variation has been shown to affect AD neuropathology and episodic memory (Rosenthal et al., 2012). *GSN* (Gelsolin) is a protein coding gene, and Gelsolin is one of the most abundant actin-binding proteins. Gelsolin binds to Aβ protein, inhibits its fibrillization, solubilizes preformed Aβ fibrils, and helps in its clearance from the brain (Yang et al., 2014). It is involved in several pathological processes, including AD (Deng et al., 2015). With these observations, the identified *ADIPOR2-ADSSL1* and *ADIPOR2-GSN* interactions could be related to the Aβ pathway.

Table 3. Seven significant SNP-SNP interaction pairs identified in the GWIS of the T-tau/Aß$_{42}$ ratio.

NO.	*SNP1*×*SNP2*	GENE	CHR	Main Effect	Interaction		Explained Variance (R Square)				
				p-value	p-value	**Corrected p-value**	Age+Gender[a]	Dx[b]	*APOE*[c]	SNP1+SNP2[d]	**SNP1*SNP2[e]**
1	rs1514061×	PLXNA4*	7	3.41E-06	8.35E-10	**0.0181**	0.01	0.164	0.129	0.015	**0.051**
	rs6467419	PLXNA4*	7	0.00167353							
2	rs1514061×	PLXNA4*	7	3.41E-06	1.97E-10	**0.0043**	0.01	0.164	0.129	0.019	**0.047**
	rs4453471	CDH13	16	0.00377003							
3	rs7303599×	ADIPOR2*	12	7.91E-05	5.76E-11	**0.0013**	0.01	0.164	0.129	0.023	**0.042**
	rs7146454	ADSSL1*	14	0.00733134							
4	rs7303599×	ADIPOR2*	12	7.91E-05	6.91E-10	**0.0150**	0.01	0.164	0.129	0.021	**0.041**
	rs167396	GSN*	9	0.00583631							
5	rs1482548×	INHBA*	7	0.007007	2.95E-10	**0.0064**	0.01	0.164	0.129	0.015	**0.038**
	rs12894119	NIN*	14	0.00867969							
6	rs9550406×	MTUS2*	13	0.00543688	1.49E-09	**0.0324**	0.01	0.164	0.129	0.010	**0.034**
	rs6471951	RLBP1L1	8	0.00604041							
7	rs211953×	CXADR	21	0.000478713	3.03E-10	**0.0066**	0.01	0.164	0.129	0.024	**0.031**
	rs4881147	PITRM1*	10	0.0015086							

The Bonferroni corrected p-values (< 0.05) and R^2 of the SNP*SNP interaction term are shown in bold.

[a] Age+Gender: Percent of variance in T-tau/Aß$_{42}$ level explained by age, gender.

[b] Dx: Percent of variance in T-tau/Aß$_{42}$ level explained by diagnosis after accounting for age, gender.

[c] *APOE*: Percent of additional variance in T-tau/Aß$_{42}$ level explained by the *APOE* genotype after accounting for age, gender and diagnosis.

[d] SNP1+SNP2: Percent of additional variance in T-tau/Aß$_{42}$ level explained by the combined main effect of SNP1 and SNP2 after accounting for age, gender, diagnosis, and the APOE genotype.

[e] SNP1*SNP2: Percent of additional variance in T-tau/Aß$_{42}$ level explained by the interaction effect of SNP1 and SNP2 after accounting for age, gender, diagnosis, *APOE* genotype, SNP1 and SNP2.

* Nearest gene proximal to the SNP.

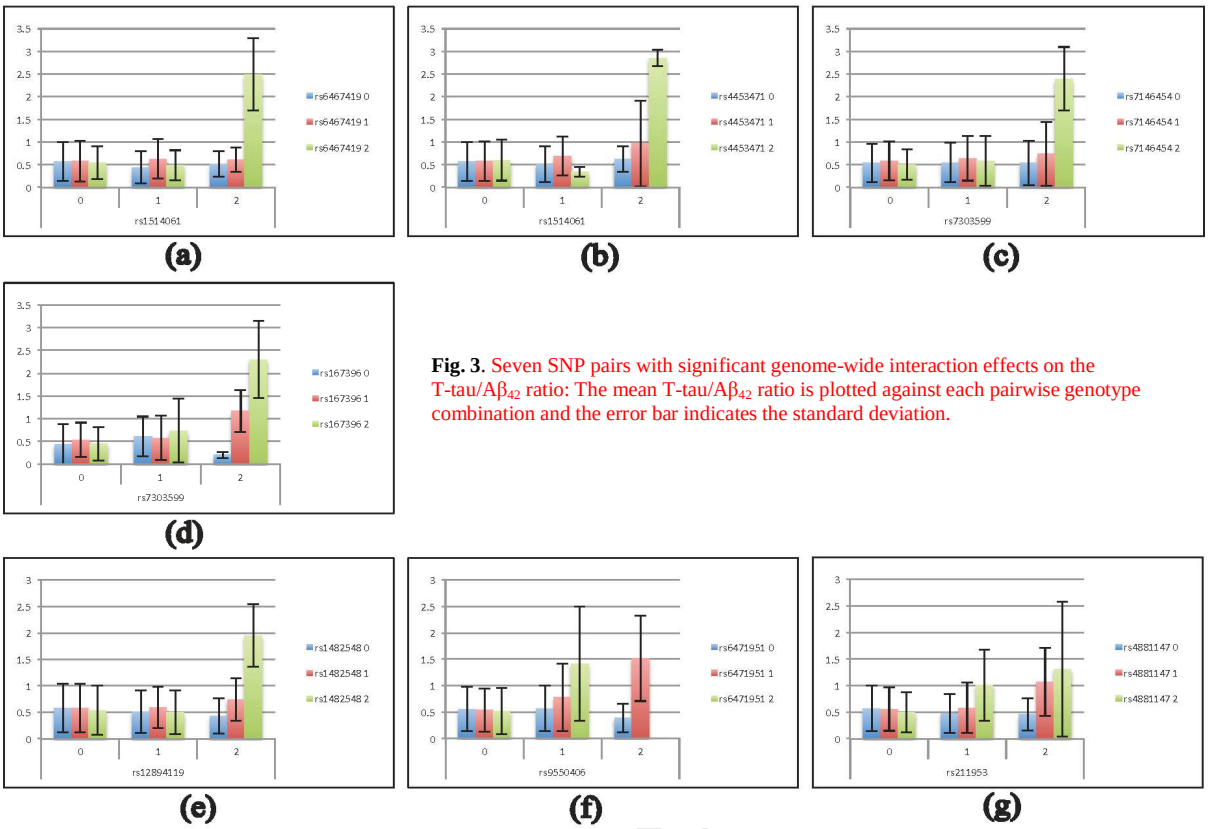

Fig. 3. Seven SNP pairs with significant genome-wide interaction effects on the T-tau/Aβ_{42} ratio: The mean T-tau/Aβ_{42} ratio is plotted against each pairwise genotype combination and the error bar indicates the standard deviation.

The protein encoded by the *INHBA* gene has been linked to neuroprotection via preventing neurons from mitochondrial dysfunction, a major cause of excitotoxicity. The corresponding process, providing protection against ischemic brain damage, could be altered in AD, or aging-related neurodegenerative conditions (Lau et al., 2015). The *NIN* gene encodes ninein (*GSK3B* interacting protein), and variants of *GSK3B* have been shown to be linked with AD and interacted with the *APOE* genotype (Izzo et al., 2013). The *MTUS2* gene encodes microtubule associated tumor suppressor candidate 2, also known as cardiac zipper protein or CAZIP. CAZIP has been shown to play a role in the development and function of the heart and nervous system in vertebrates (Du Puy et al., 2009). *PITRM1* is responsible for significant Aβ degradation, and the impairment of its activity results in Aβ accumulation (Brunetti et al., 2016). The possible mechanisms behind *INHBA-NIN*, *MTUS2-RLBP1L1*, and *CXADR-PITRM* interactions warrant further investigation.

In summary, some of the genes identified in our GWAS and GWIS have shown interesting associations with tauopathies and/or amyloid pathology related to AD from prior knowledge of current literatures, such as *APOE*, *APOC1*, *TOMM40*, *LRP6*, *S100B*, *DLG2*, *CNTNAP5*, *PLXNA4*, *CDH13*, *ADIPOR2*, *ADSSL1*, *GSN*, and *PITRM* genes (see Supplementary Tables s4a and s4b). Supplementary Tables s4a and s4b showed that these 13 genes were reported in previous genomic, cell culture, mouse model and biomarker studies, and shown to be significantly associated to CSF Aß42, CSF T-tau or other AD endo-phenotypes. However, in this work, only *APOE*, *APOC1*, *TOMM40* genes showed significant associations to the CSF Aß$_{42}$, T-tau and T-tau/ Aß$_{42}$ levels, other genes were not identified by GWAS and GWIS of Aß$_{42}$ alone or T-tau alone (Supplementary Table s2 and Table s3). This indicates that, when CSF Aß$_{42}$ alone and T-tau alone show less power for detecting the risk variants, the T-tau/ Aß$_{42}$ ratio has the potential to serve as a more powerful quantitative trait to identify significant variants. In addition, our study also revealed numerous SNPs and SNP-SNP pairs that had not yet been associated with AD pathology, which warrant further investigation or replication in future studies.

5. Conclusions

Aimed at studying a major AD biomarker as phenotype, we performed GWAS and GWIS to detect the main genetic effects as well as SNP-SNP interaction effects on the CSF T-tau/Aß$_{42}$ ratio. The single-marker analysis replicated the *APOE*, *APOC1* and *TOMM40* genes, which are previously confirmed AD risk genes. We also identified 14 additional loci within or proximate to *LRP6*, *S100B*, *DLG2*, *CNTNAP5*, *B3GALT2*, *FBP1*, *ITGA8*, *ATP5F1*, *LPAR3*, *DAPK2*, *DBX1*, *AADACL1*, *SGIP1*, and *ARHGAP28*. The two-marker interaction analysis identified a number of novel interaction findings, which showed strong associations with the T-tau/Aß$_{42}$ ratio. These were interactions between *PLXNA4* and *PLXNA4*, between *PLXNA4* and *CDH13*, between *ADIPOR2* and *ADSSL1*, between *ADIPOR2* and *GSN*, between *INHBA* and *NIN*, between *MTUS2* and *RLBP1L1*, and between *CXADR* and *PITRM1*. The effects of SNP-SNP interactions showed high-level statistical significance, while the corresponding single-marker effects were marginal. SNP-SNP interaction effects may help address part of "miss heritability".

Our genome-wide association study and interaction study have the following strengths. (1) Continuous quantitative trait

T-tau/Aβ_{42} can not only gain higher statistical power, but also contribute to detecting potential risk variants related to T-tau and/or Aβ_{42} at the same time. (2) Five values (1-5) indicating CN, SMC, EMCI, LMCI and AD respectively, provide a rank ordered spectrum of the AD progression. (3) In this study, both GWAS and GWIS consider age, gender, and clinical diagnosis as covariates. In the post hoc linear regression analysis we included confounding factors *APOE ε4* allele (rs429358) on top of the above three covariates, and so provided more accurate estimate of the interaction effects on CSF T-tau/Aβ_{42} ratio.

The limitations of our study are as follows: (1) We examined 22 million SNP-SNP pairs and conducted an exhaust test among the SNPs. More effective and efficient strategies remain to be developed. (2) To control for potential false positives of the GWIS findings, we used two methods. One is the Bonferroni method, which corrects for multiple comparison by using a threshold of α/n and is well-known to be a conservative approach. Another method used in the work is the cell-size criterion, which excludes rare genotype combinations to avoid potential false positives. In this work, we set α=0.05 and n= 21,717,345 for Bonferroni correction; and for the cell-size criterion, all the cell sizes in the 3-by-3 contingency table are required to be either more than 5 or equal to 0. There are 307 pairs SNPs passed the first threshold, and subsequently only 7 pairs among 307 pairs passed the second threshold for further study. Although these are relatively stringent criteria for controlling false positives, future replication studies are required to confirm the identified interaction signals. (3) We performed the GWAS and GWIS of CSF T-tau/Aβ_{42} ratio using data-driven method. Future studies could utilize prior biological knowledge, such as biological networks, pathways databases, special tissues and other functional annotation data, to enhance statistical power and improve biological interpretability. (4) Future studies are necessary to replicate and validate the findings in independent datasets, and to uncover potential mechanisms underlying tagged by the identified SNPs and genes in our study.

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Disclosure Statement

The authors have no actual or potential conflicts of interest including any financial, personal, or other relationships with other people or organizations that could inappropriately influence (bias) our work.

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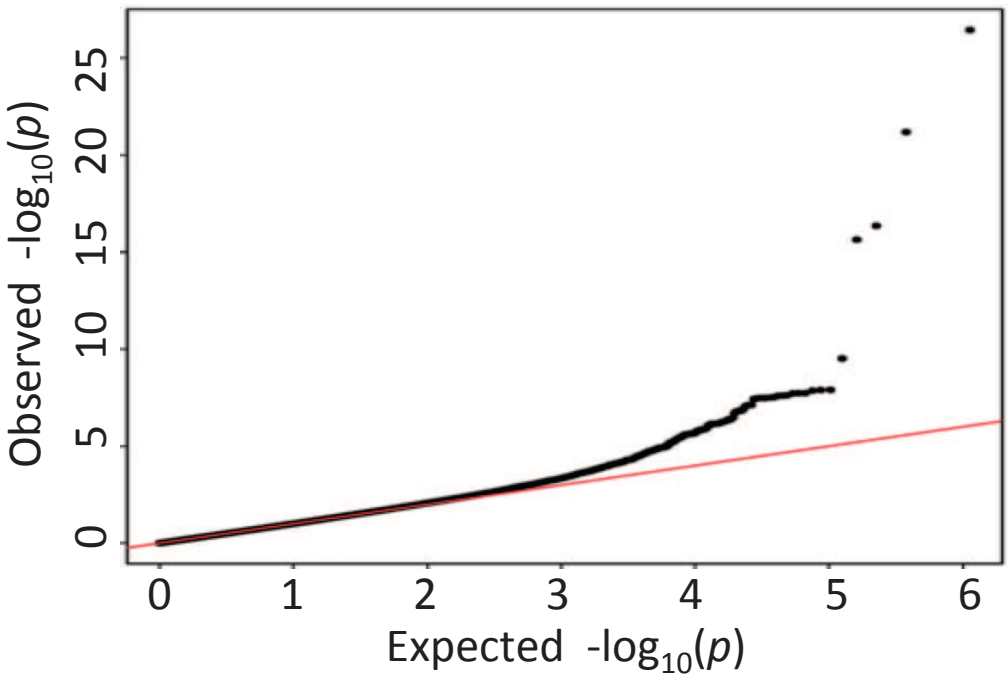
Observed -log10(p)
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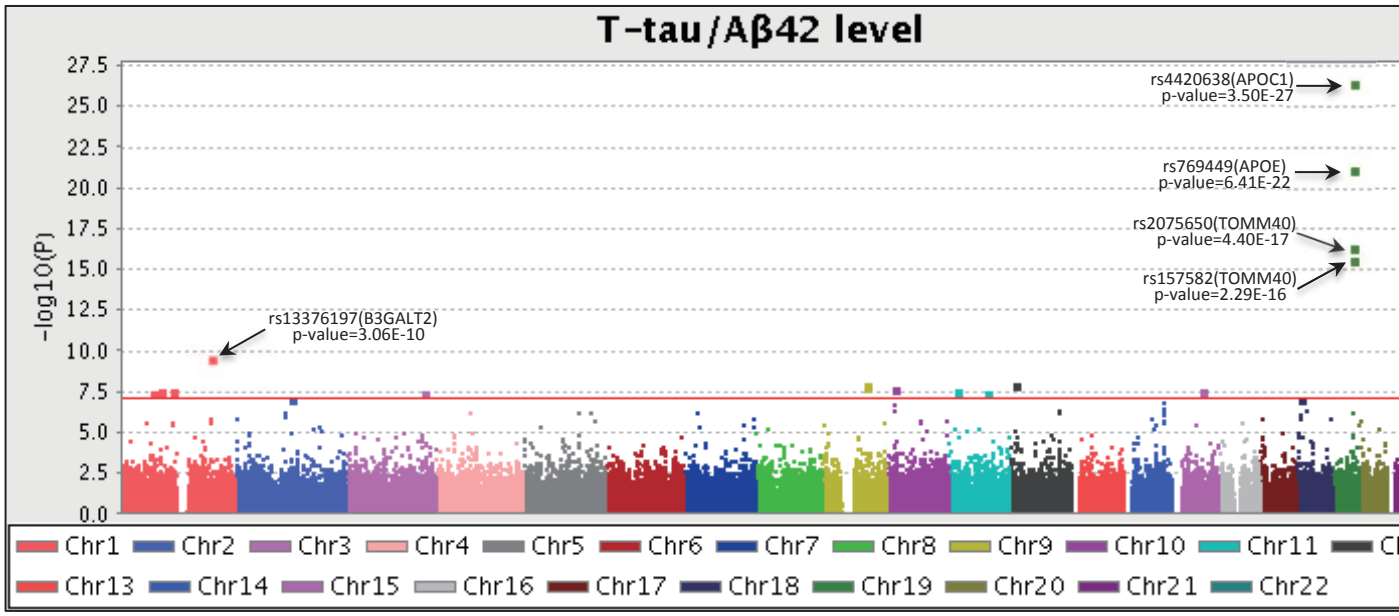
10
15
20
25
0
1
2
3
4
5
6
Expected -log10(p)



Highlights

- SNP-SNP interaction effects may help address part of "miss heritability".
- GWAS and GWIS of T-tau/Aß$_{42}$ ratio were performed on the landmark ADNI database.
- This is the first GWIS on the quantitative trait of the T-tau/Aß$_{42}$ ratio.
- The interaction results had marginal main effects but explained relatively high level T-tau/Aß$_{42}$ variance.